DESCRIPTION OF PLATE.

Figs. 1 to 7 inclusive, are all photographed with Spencer 4 mm. objective and 10 × eyepiece.

Fig. 1.—Transverse section of Sakel hair, possibly accidentally pressed, and slightly swollen with sub-critical strength sulphuric acid ; showing radial (spiral) cracks.
Fig. 2.—Transverse section of same cotton as fig. 1. Section lightly pressed, then swollen with critical strength sulphuric acid ; showing growth rings.
Fig. 3.—Ordinary Sakel hair mounted in liquid paraffin, untreated. (Denham and Harland's method.)
Fig. 4.—Hair of Sakel cotton boiled in dilute HCl, and pressed.
Fig. 5.—Hair of Sakel cotton pressed heavily in 8 per cent. caustic soda.
Fig. 6.—Hair pressed in naphthamine blue. Spiral structure entirely due to pressure only.
Fig. 7.—Hair of Sakel cotton pressed lightly in an adjusted mixture of cuprammonium and soda.
Figs. 8 and 9 are photographed with Spencer 16 mm. objective only, and 10 × eyepiece.
Fig. 8.—Hair swollen with calcium thiocyanate, showing double thread spiral.
Fig. 9.—Hair of Sakel cotton stained with iodine, but not otherwise treated ; showing double pit spirals.

Observations on the Distribution of Fat-Soluble Vitamines in Marine Animals and Plants.

By Johan Hjort, D.Sc., For.Mem.R.S.

(Received April 27, 1922.)

(From the Biochemical Laboratory, Cambridge.)

The Norwegian fishery investigators have for many years been engaged in the study of the growth of fish, mainly the herring and the cod. By means of microscopical study of the scales of the fish it has been possible to determine the age of each individual fish, and by means of the assumption, which has been verified within certain limits, that there is a proportion between the length of the scale (l_s) and the length of the fish (l_f) (*i.e.*, $l_s/l_f =$ constant), it has been possible to calculate the "growth curve" of the fish in different years of its life, and in different seasons of the year. The results of this work, which have been summarised up to the year 1914,* proved that the growth of the said fish in the Norwegian waters was confined to a few spring—and summer—months only, and that the growth of the fish entirely ceases during the winter season. Corresponding to the summer and winter zones, which are to be seen on the scales of the fish, we find a periodicity in the increase of length and weight of the fish, and a change in the quality of the fish at the different seasons of the year.

---

* Johan Hjort, "Fluctuations in the Great Fisheries of Northern Europe," 'Rapports et Procès-verbaux du Conseil International,' vol. 20, Copenhagen (1914).

The changes in the "quality" of the fish were very early observed to be associated with the changes occurring in the content of fat. Chemical analyses were made by Mr. H. Bull of the fat contents of herrings and sprats at different months of the year,* and from these observations the conclusion was drawn that "the supply of fat increases during the summer and is consumed during the winter, while water is excreted in the summer and assimilated in winter. During the winter, part of the dry matter in the system is consumed and replaced by water, so that no great loss in weight is apparent. The quality of the fish, however, is considerably affected."

In the herring this is very apparent, the fat being deposited in a special peritoneal fat organ (by Norwegian fishermen called the "ister"), which is especially developed in summer and in the young year classes which have not yet developed their generative organs. As stated in the paper, the contents of the fat organ "varies with the age of the fish, the development of the genital organs, in particular, being a factor of great significance. In addition to this, the quality of ister varies greatly according to the time of year; this applies not only to the ister itself, but also to the contents of fat on the whole as shown by chemical analysis."

In the cod the changes in quality are most easily demonstrated by the inspection of the size of the liver, the contents of which, so far as some 50 per cent. is concerned, consist of the "cod-liver oil." Besides by chemical analyses, the liver has been studied by means of weighings. The livers of cod of different sizes (in groups of different lengths) have been weighed at different times of the year, and it has been found that the liver of the full-grown cod during the summer season, when the fish was feeding, weighed no less than three times as much as in the winter during the spawning season. "The greatest 'depreciation' in the quality of the cod takes place during their stay on the Lofoten Banks, where the genital organs arrive at full maturity and spawning takes place."†

These observations naturally led to a consideration of the problem of the conditions which determine the great changes in the growth of the fish themselves, and particularly in the organs in which the reserve material (fats) are stored. A comparison between the amount of fat in the sprat of the west coast and the mean surface temperature of the sea for the different months of the year* showed a remarkable correspondence for the spring and early summer months, *i.e.*, the amount of fat increases in close correspondence with the rise in temperature in the spring, but this relationship does not continue during the late summer and autumn, thus indicating that the temperature cannot be considered as the only influence concerned.

---

* See *loc. cit.*, Chapter V.

† *Loc. cit.*, p. 195.

The rise of temperature in the sea during the months of spring is of course in northern waters accompanied with a great number of other phenomena, viz., the increase of the intensity of light, the increased addition to the sea-water of masses of fresh water from the continents carrying with them inorganic and organic substances from the land and the seashore. As a result of these events an "eruptive" development of plant life takes place, and shortly afterwards plankton animals (*i.e.*, copepods) develop and bottom animals begin to increase their growth.

While the development of the plants and of the small animals which directly prey on them may seem easy to understand as a result of the seasonal changes of the spring, the increase of the growth of larger animals like the cod, the prey of which exist all the year round, seems much more difficult to explain. The problem, therefore, seemed worth examination, whether the decrease of growth in the autumn and the increase of growth in the spring were connected with the availability of certain specific kinds of food, in other words if there could be found in nature a variation of chemical qualities in the food of the animals.

The fact that the changes in the quality of the animals during the different seasons have been found to be in such a close connection with changes in their contents of fats raised the desirability of investigating the distribution of the fat-soluble vitamines in organisms of the sea, since these vitamines have been found to have such a great influence on the growth of animals.

During my work at the Biochemical Laboratory of the University of Cambridge, Prof. F. G. Hopkins had the great kindness to offer me the opportunity of conducting experiments on such vitamine problems, and allowed me for this purpose to have the benefit of the organisation for experiments on rats which he has established at his laboratory. For the purpose of such experiments during the summer 1921 I made a collection of different material of plankton and bottom animals from Norwegian waters. This material was preserved in alcohol, and it was extracted in the laboratory (under anaërobic conditions) with alcohol and ether, in order to extract the oils for the experiments. This plan, however, had no success. The fats obtained in this way were in the form of waxes, and it was soon realised that for some reason they were unsatisfactory for nutritional experiments and gave ambiguous results. Other methods had therefore to be applied.

---

* *Loc. cit.*, p. 173.

In the spring of 1922 material was, therefore, collected partly in a fresh or sterilized condition, partly in alcohol. In co-operation with my assistant, Dr. Axel Palmgren, a different method was developed for the extraction of the oils.

The fresh material was first minced, then dried in thin layers on filter paper in the constant-temperature room of the laboratory (at 37°). The dried substance proved in all experiments as effective as fresh substance, and it could therefore be applied for the purpose of extraction by acetone or benzene. The filtrates (from three to four extractions) were evaporated *in vacuo*, the last part of the solvent evaporated off in a water bath at 90°–100° in a high vacuum for 15 minutes, till the remaining oil no longer smelt of benzene. The oil was then dissolved in olive oil, and the mixture given to rats by a counted number of drops, 100 drops making 3 c.c. of oil. At a specific gravity of 0·9, one drop would then have a weight of 2·7/100, or 27 mgrm. If one part of the oil had been dissolved in seven parts of olive oil, eight or sixteen drops were given to each rat daily, in order to give the rat one or two drops of the oil under experiment. In the following "one drop" means one drop of the oil under experiment. The oil was not mixed with the food, but administered by means of a pipette kept just over the mouth of the rat, which was laid on its back in the left hand. This method of feeding was recommended to me by Prof. E. Poulsson, of the University of Christiania.

The experiments are all of a preliminary character, and had mainly the purpose of exploring the distribution of fat-soluble vitamines, for future more detailed and more quantitative studies. In spite of their preliminary character, it has been thought useful to publish the following observations, as they may stimulate other workers to investigate this field.

Marine Plants.

As a preliminary investigation, it seemed to me of interest to try some green algæ as representatives of the vegetation along the shore and some samples of the first growth of diatoms in the coast waters during spring.

Green algæ (*Ulva lactuca* and *Codium tomentosum*) were kindly collected for me at the Marine Biological Station at Port Erin by H. H. Thomas and S. H. Wadham, of the Botany School of Cambridge. The algæ were brought to us in fresh condition, and given partly in fresh and partly in dried condition to rats which for some time had been on a diet free from fat-soluble vitamines, but containing vitamine B. and other essentials. The three curves (figs. 1 and 2*) all show a marked increase in the weight of the rats when given the seaweed. Fig. 1, the lower curve, represents the growth on fresh seaweed, kept in cold store. The increase is not so marked as the two other curves showing the effect of dried seaweed. This produced an enormous increase in the weight. The dried seaweed was added to the food as a fine green powder and was thoroughly mixed with the food.

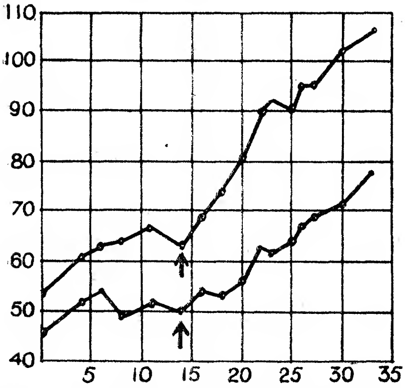

Fig. 1.—Lower curve, fresh ulva, *ad lib.* Upper curve, dried ulva, 1 grm. per day.

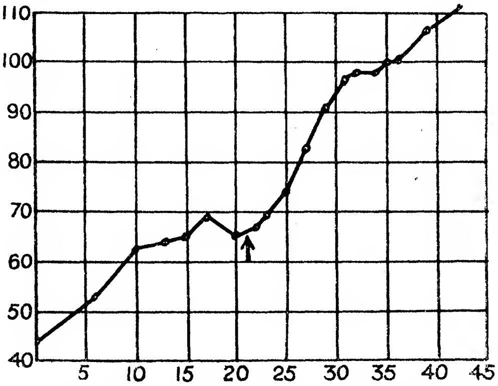

Fig. 2.—Dried ulva, 1 grm. per day.

In order to see if the growth-promoting factors of the ulva were soluble in its fats, dried powder of ulva was extracted with acetone and the oil given (1 or 2 drops a day) to the rats. The resulting growth-curves may be seen

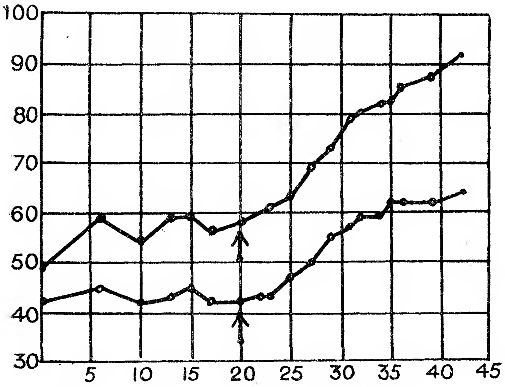

Fig. 3.—Extract (by acetone) of ulva, two drops a day.

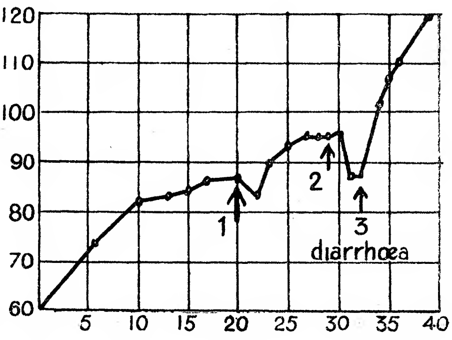

Fig. 4.—Point 1, added 1 gr. sterilised diatoms per day. Point 2, 1·5 grm. dried diatoms per day. Point 3, 2 drops benzene extract of diatoms per day.

---

* In all the figures the abscissa gives the number of days of experiment, the ordinate the weight in grams.

from fig. 3. They demonstrate a marked increase in growth of the rats from the moment when the oil was given.

Samples of *Diatoms* were collected for me by my friend, Prof. H. H. Gran, and Mr. E. Lea on board a Norwegian research vessel. The samples consisted of a pure " Diatom-plankton," as this may be found in the sea in the spring before the animals have started their development. The rats fed on these diatoms showed first a sharp increase of growth, and then, after a short time, a marked decline. The idea suggested itself that the diatoms, which contain so much silica, may be very unsuitable as food for a mammal, and diarrhœa was also observed in one case (see fig. 4). In some cases, two drops a day of green oil, extracted by benzene from the dried mass of diatoms, gave at once a marked increase, even in the case of animals which had first been fed on the dried material (fig. 5). Although these experiments, which were hampered by rather scanty material, do not give such a marked and striking growth as the curves of the seaweed, I have no doubt that an increased growth has been demonstrated as a result of addition to the basal diet of the rats of oils extracted from diatoms.

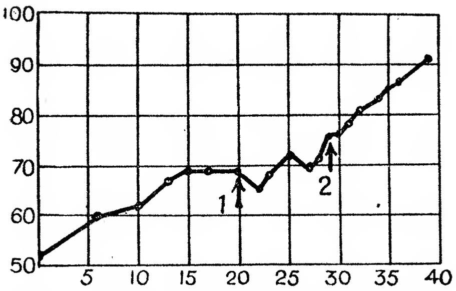

Fig. 5.—Point 1, *ca.* 0·8 grm. dried diatoms per day. Point 2, 2 drops extract per day.

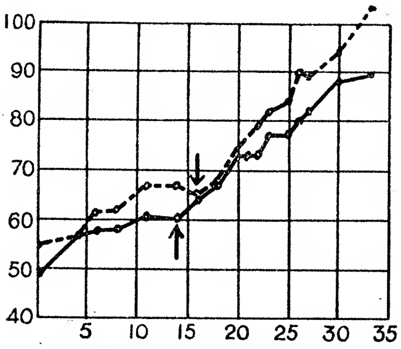

Fig. 6.—Fresh shrimps *ad lib.*

Shrimps and Prawns.

Fresh shrimps (*Crangon*) and fresh prawns (*Pandalus borealis*) were minced and given fresh as an addition to the basal diet of several rats. Figs. 6 and 7 show a marked increase in the weight of the rats shortly after the addition of these substances to their diet.

Dried powder was extracted by benzene, the extracted oil (two drops a day) had in several cases no effect, a fact which I am not able to explain and which must be left for future investigation.

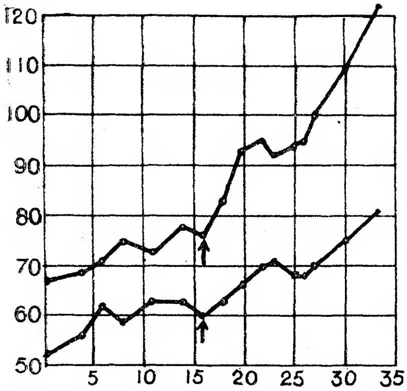

FIG. 7.—Fresh prawns *ad lib.*

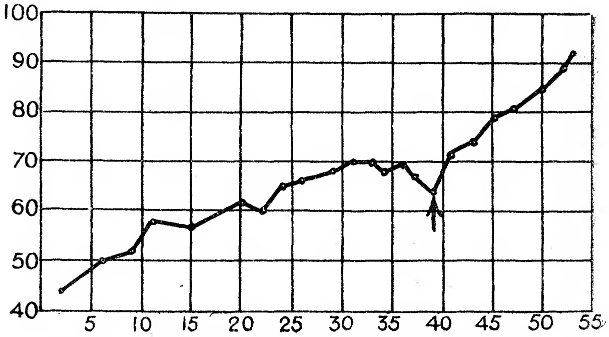

FIG. 8.—Four drops of oil from roe of herrings.

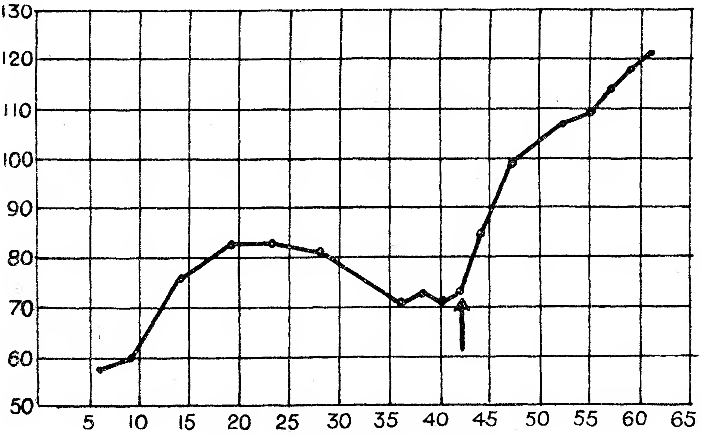

FIG. 9.—Fresh cod roe *ad lib.*

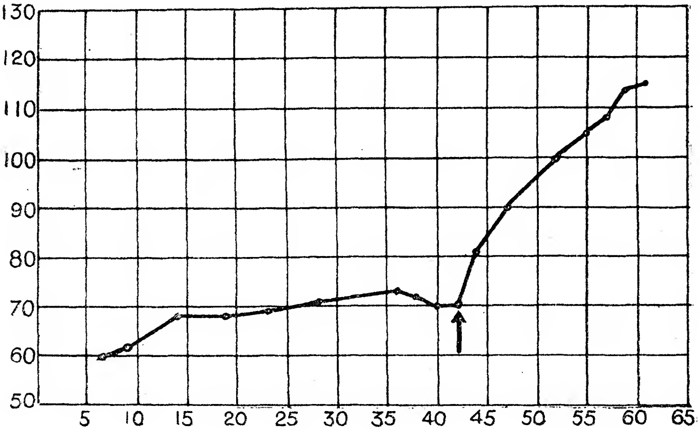

FIG. 10.—Fresh cod roe, 2 grm. per day.

Herring and Cod.

I have mentioned in the introductory remarks above that the fat organ of the herring and the liver of the cod both decrease in weight at the same time as the ovaries of the female animal increase in weight. An examination of the contents of fats and of fat-soluble vitamines of the ovaries (the roe) would therefore seem desirable. Fig. 8 shows the effect of addition of oil extracted from the ovaries of herrings.

Fresh cod roe (figs. 9 and 10) in a dose of 2–3 grm. per day produced an immediate and rapid increase in the weight of rats. Dried roe, prepared in Norway for commercial purposes (1 grm. added per day), had (figs. 11 and 12) just as marked an influence; and this fact must, like the effect of the dried ulva, be considered as very important, since it proves that the process of drying fish products had no observable destroying effect on the growth-

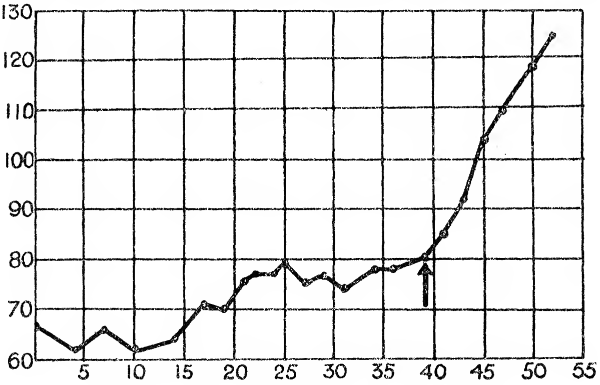

Fig. 11.—Dried cod roe, 1 grm. per day.

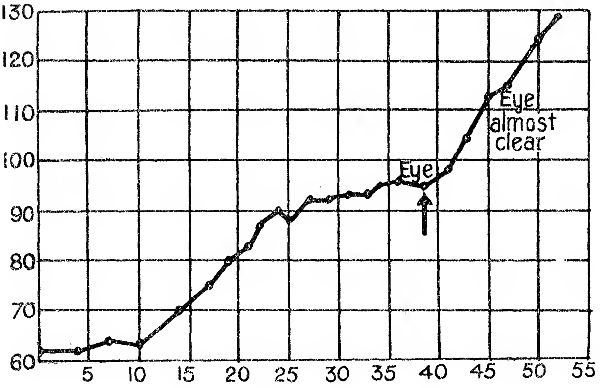

Fig. 12.—Dried cod roe, 1 grm. per day. When first given one eye very bad, after some days almost clear.

promoting factors. Drying of fish and fish products plays a great *rôle* in the fishing industry of different countries, and it is therefore of practical value to know that the process does not deprive the products of these valuable qualities.

Extraction of the powdered dry roe by means of benzene produced a clear but thick oil, different from the wax, which was obtained by extraction with alcohol and ether. The effect of one drop daily of this oil is illustrated by the curves on fig. 13. In one rat, which had developed the eye disease (keratomalacia) characteristic of cases upon a diet deficient in the fat-soluble vitamine, this disease was cured after the addition of the oil of the roe, but this rat did not later on show any increase in its growth.

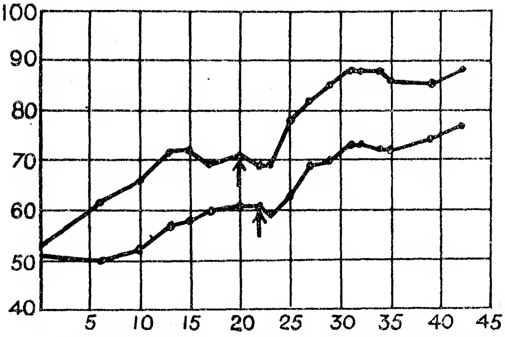

Fig. 13.—Benzene extract of dried cod roe, 1 drop per day.

The fact that oils extracted from marine plants have been proved to have a very strong effect on the growth of rats fed on a diet deficient in fat-soluble vitamines seems to indicate the working hypothesis that fat-soluble vitamines in the sea arise in plants in a similar way to that proved to be the case in plants on land. It seems, further, natural to assume that all marine animals, directly or indirectly, obtain these substances from the plants, and that the marine animals, like other animals studied, are unable to synthesise these substances. An important programme for future work seems then to suggest itself, viz., to determine the quantitative distribution of fat-soluble vitamines in different types, and in them during different seasons of the year. It may be possible by such studies to approach the important problems of the influence of the food of animals on that periodicity in their growth which is so remarkable in Northern waters. The present investigations seem to indicate the problem to ascertain how far the production of fats and fat-soluble vitamines in the plants during spring and early summer months will have to be regarded as the store on the magnitude of which the growth of animals is dependent in Northern latitudes. In Southern latitudes the growth of fish has been found much more uniform throughout the year; the changes in the production of plants are there not so marked as in the high Northern latitudes.

Dr. Palmgren and I intend, if circumstances permit, to continue the investigations towards the solution of these problems at the Biological Laboratory of the University of Christiania.

On Blood-Platelets: their Behaviour in " Vitamin A " Deficiency and after " Radiation," and their Relation to Bacterial Infections.

By W. Cramer, A. H. Drew, and J. C. Mottram.

(Communicated by Prof. W. Bulloch, F.R.S. Received March 28, 1922.)

(From the Laboratories of the Imperial Cancer Research Fund and from the Radium Institute.)

Introductory. General Effects of Fat-soluble Vitamin Deficiency.

When the fat-soluble vitamin A is withheld from the diet of a rat, the general condition of the animal differs from that resulting from a deficiency of the water-soluble B vitamin. In the latter case the animal ceases to increase in weight almost at once, and then begins to decline. There is a progressive fall in the temperature. The animals always die within a comparatively short time—two months—and are then found to be in a state of profound emaciation, as if they had received no food at all. There is no obvious sign of disease or of an infection. For the sake of convenience, we will designate briefly this condition by the term " marasmus."

The withholding of the fat-soluble vitamin A alone affects different individual rats in a manner as varied and indefinite as the conditions obtained by a deficiency in the water-soluble B are constant and definite. When a young rat is kept on a diet from which the fat-soluble vitamin A is absent, the increase in weight may cease almost at once, or it may continue to increase in weight for many weeks, and almost as rapidly as on a diet containing vitamin A, although eventually its growth will come to a standstill before the full normal size of an adult rat has been reached. We shall, for the sake of convenience, describe these two extremes as the " acute " and the " chronic " effect on growth respectively. Eventually the